Author's response to reviews

Title: Ruscogenin ameliorates diabetic nephropathy by its anti-inflammatory and anti-fibrotic effects in streptozotocin-induced diabetic rat

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Author's response to reviews: see over
Reply to Editor

Manuscript Number: 18779597491111005
Title: Ruscogenin ameliorates diabetic nephropathy by its anti-inflammatory and anti-fibrotic effects in streptozotocin-induced diabetic rat
Authors: Hung-Jen Lu, Thing-Fong Tzeng, Shorong-Shii Liou, Sheng Da Lin, Ming-Chang Wu and I-Min Liu

Dear Editor,

We appreciate your kind support of our presentation and thanks for your kind agreement. The point-by-point replies for each comment are indicated in the letter to the reviewers. The changes of the revision are highlighted in red. We sincerely hope that this revised version will be suitable to meet your excellent standards of acceptance. Thanks for your kind consideration.

Best regards and hope to have your final decision soon,

Respectfully,
Prof. I-Min Liu,
Reply to Reviewer’s (Referee 1) Comments

Manuscript Number: 1877959749111005
Title: Ruscogenin ameliorates diabetic nephropathy by its anti-inflammatory and anti-fibrotic effects in streptozotocin-induced diabetic rat
Authors: Hung-Jen Lu, Thing-Fong Tzeng, Shorong-Shii Liou, Sheng Da Lin, Ming-Chang Wu and I-Min Liu
Reviewer: Juei-Tang Cheng

In the discussion Authors should explain if and how their results can be applied to humans. Are there already studies in humans? If yes, which dosage of zerumbone was given?

Reply

Using a metabolism coefficient of 6.25 to convert the effective daily oral dose of ruscogenin for rat (3.0 mg/kg) into a clinical dose, assuming an average adult body weight of 60 kg, we estimated a daily oral dose of zerumbone for humans to be approximately 32 mg. Due to different metabolism in humans and rats, the results come from rat studies cannot generalize to human. The placebo controlled human studies are required to find the usability of ruscogenin in human indication for DN. Please find it in the 2nd paragraph on page 15. We wish this change would be satisfactory.

The changes of the revision are highlighted in red. We hope that this revised version of our work will meet your high standards for acceptance. Also, I wish to express my warmest thanks to you again. Your kind agreement of acceptance will be sincerely appreciated.
Reply to Reviewer’s (Referee 2) Comments

Manuscript Number: 1877959749111005
Title: Ruscogenin ameliorates diabetic nephropathy by its anti-inflammatory and anti-fibrotic effects in streptozotocin-induced diabetic rat
Authors: Hung-Jen Lu, Thing-Fong Tzeng, Shorong-Shii Liou, Sheng Da Lin, Ming-Chang Wu and I-Min Liu
Reviewer: LEE-TIAN L. T. CHANG

Dear distinguished referee:
Thank you very much for reading this manuscript and the helpful comments. The revision has been amended according to your kind suggestions as follows:

**Major Compulsory Revisions**

**Reply 1**
(1) According to your kind suggestions, the scale bars have been inserted in Fig. 1, 2, 4&5. Furthermore, the dpi in the indicated figures has been improved. Besides, the arrowhead was used to point out the positive areas in the indicated figures. We honestly wish it would be suitable to meet your requirement.

(2) Actually, the tissue slices in figure 1 were stained with H&E. We have been corrected the typing mistakes in Methods section (line 4 on Page 8) and legend for Figure 1 (Page 21).

(3) ICAM-1 is a known important downstream inflammatory factor whose overexpression promotes inflammatory cells, including mononuclear macrophage infiltration into glomeruli and renal interstitium, as well as accelerates glomerular sclerosis in diabetes (Nephron 79:91, 1998). In addition, MCP-1 is involved in the inflammatory response by activating the macrophages from the circulation to the local kidney and then promote the expression of other proinflammatory cytokines to augment the accumulation of extracellular matrix (J Diabetes Complications 17:11, 2003). Using accumulation of ED-1 as a marker of macrophage activation, we have demonstrated that increased macrophage activation in the glomeruli of kidney tissue from STZ-diabetic rats, which was reduced by ruscogenin (Figure 2). The higher levels of renal MCP-1 and ICAM-1 proteins in STZ-diabetic rats were also lowered.
by ruscogenin treatment (Figure 4). In could be reasonable considered that the inhibitory effect of ruscogenin on MCP-1 and ICAM-1 may be due to the decreased infiltration of monocytes/macrophages. Please find it in the last 4 lines of the first paragraph on Page 13. Actually, we agreed your opinion that the double stain on ED-1 cells and MCP-1/ICAM-1 nephron cells in Figure 2 and 4 will confirm the anti-inflammation effect of ruscogenin. We will arrange the further study to support the results in the future. We are hopeful that this clarification is acceptable and appreciate this helpful comment.

Reply 2
We totally agreed your opinion that an advanced mechanism study will help us to understand the potency of ruscogenin on clinical usage. Actually, the renal expression of inflammatory cytokines such as TNF-α, IL-6 and IL-1β were demonstrated to increase in diabetes, contributing to the development of DN (J Am Soc Nephrol 19:433, 2008). Along with the effects on macrophages, there was a reduction in the upregulated protein expression of TNF-α, IL-6 and IL-1β from kidneys of STZ-diabetic rats receiving ruscogenin treatment (Figure 3). Thus, we believe that the anti-inflammatory effects of ruscogenin, through the inhibition of macrophage infiltration, might provide a renoprotective effect in the STZ-diabetic model. Please find it in the last 7 lines on Page 12. We are hopeful that this clarification is acceptable and appreciate this helpful comment.

Reply 3
It is widely known that activation of PPARγ attenuates the NF-κB-mediated transcriptional activation of proinflammatory genes (Nat Rev Immunol 2:748, 2002). Studies have also demonstrated that PPARγ agonist exerts a renoprotective effect through an anti-inflammatory mechanism in DN (J Diabetes Complications 23:124, 2009). Thus, rosiglitazone was used as positive control to compare the effect of the renoprotective effect of ruscogenin. However, ruscogenin cannot lower hyperglycemia and HbA1c in STZ-diabetic rats. A test to clarify the role of ruscogenin on the PPARγ receptor or evaluate the influence of ruscogenin on the action of insulin seems to be helpful in the identify the clinic potential of this compound. We will arrange further studies to solve the issues in the future. We are hopeful that this interpretation is acceptable and appreciate this helpful comment.
Minor Essential Revisions

Reply 1
The typing error has been corrected according to your indication. Please find it in line 3 of the 2nd paragraph on Page 13. Thank you very much.

Reply 2
Word sizes of each figure have been improved following your kind recommendation. Thank you so much.

Reply 3
Considering the effects of 3.0 mg/kg/day ruscogenin on the improvement of renal function in STZ-diabetic rats were closed to those produced by rosiglitazone, the kidney in STZ-diabetic rats receiving 3.0 mg/kg/day ruscogenin treatment was further isolated to delineate the potential underlying mechanisms. We indicated in the last 4 lines on the first paragraph of Page 11. We are hopeful that this clarification is acceptable and appreciate this helpful comment.

The changes of the revision are highlighted in red. We hope that this revised version of our work will meet your high standards for acceptance. Also, I wish to express my warmest thanks to you again. Your kind agreement of acceptance will be sincerely appreciated.